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DELHI TECHNOLOGICAL UNIVERSITY

Department of Biotechnology

Shahbad Daulatpur, Delhi – 110042

B.Tech Biotechnology — Final Year Thesis

Structural Insights into LasR–QslA Complex Inhibition: A Virtual Screening and Molecular Docking Analysis of Natural Sesquiterpenes

Submitted by:

Shivani Kaushik

Roll No: [Your Roll Number]

Under the Supervision of:

Dr. Kriti Bhandari

Assistant Professor, Department of Biotechnology

Delhi Technological University

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ABSTRACT

SBDD is now recognized as an effective computational method in the design of new therapeutics- both in the presence of a detailed 3D crystal structure of the target protein. In this thesis LasR-QslA complex (PDB ID: 4NG2) of.

Pseudomonas aeruginosa was taken as structure drug target. We only worked with a structural and computational approach, exploiting the precise geometry of the autoinducer-binding space of the complex to discover a more specific inhibitor.

A curated library of 2,500 natural phytochemicals — filtered by physicochemical drug-likeness criteria — was screened using the PyRx-integrated AutoDock Vina engine. The grid box is precisely focused on the coordinates of the co-crystallized native ligand 3-oxo-C12-HSL in the LasR LBD. Validation of the docking protocol was performed by redocking of beta-caryophyllene, which exactly reproduced the published affinity value (-5.5 kcal/mol) — confirming that the computational setup was methodologically sound [4,6].

The top-ranked compound was Glomeremophilane B (PubChem CID: 139589801), with a binding affinity of -7.9 kcal/mol—thermodynamically superior to both the native autoinducer 3-oxo-C12-HSL (-5.2 kcal/mol) and the reference beta-caryophyllene (-5.5 kcal/mol). Post-docking 2D and 3D interaction analysis in BIOVIA Discovery Studio revealed that Glomeremophilane B forms six conventional hydrogen bonds with critical polar residues TRP75, TRP76, THR76, and GLN103 of the LasR binding pocket—a dense polar engagement pattern that is structurally far superior to the single H-bond interaction of the native ligand [1].

This thesis primarily argues that the eremophilane sesquiterpene scaffold of glomeremophilane B achieves the ideal pharmacophoric fit for the LasR-QslA autoinducer pocket due to its rigid ring geometry, oxygen-bearing substituents, and multi-point hydrogen bonding capacity. This is a purely structural and mechanistic perspective presented as a 30-page computational study.

Keywords: LasR–QslA, 4NG2, Structure-Based Drug Design, AutoDock Vina, PyRx, Glomeremophilane B, Virtual Screening, Sesquiterpene, Molecular Docking, Pharmacophore, Hydrogen Bond Network, Interaction Fingerprint, Binding Pocket Geometry, Conformational Fit

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LIST OF ABBREVIATIONS

ADME — Absorption, Distribution, Metabolism, Excretion

AHL — N-Acyl Homoserine Lactone

BBB — Blood-Brain Barrier

BIOVIA DS — BIOVIA Discovery Studio

CYP — Cytochrome P450

GI — Gastrointestinal

H-bond — Hydrogen Bond

LBD — Ligand Binding Domain

LuxR — Luminescence Regulator (LuxR-family)

MD — Molecular Dynamics

MM-GBSA — Molecular Mechanics Generalized Born Surface Area

MM-PBSA — Molecular Mechanics Poisson-Boltzmann Surface Area

PDB — Protein Data Bank

PyRx — Python Prescription (Virtual Screening Platform)

QS — Quorum Sensing

QSI — Quorum Sensing Inhibitor

QslA — Quorum Sensing-Like Antiactivator

RCSB — Research Collaboratory for Structural Bioinformatics

RMSD — Root Mean Square Deviation

SBDD — Structure-Based Drug Design

SBVS — Structure-Based Virtual Screening

SDF — Structure Data File

SMILES — Simplified Molecular Input Line Entry System

TPSA — Topological Polar Surface Area

UFF — Universal Force Field

CHAPTER 1: INTRODUCTION

1.1 Structure-Based Drug Design (SBDD): Foundation of Computational Drug Discovery

Drug discovery was traditionally an extremely time-intense and expensive procedure. In the past, researchers would physically synthesize literally thousands of compounds and test each one experimentally—a random trial-and-error approach that could take decades to bring a single medication to market. Today, computational biology has fundamentally changed the game.[1] Structure-Based Drug Design (SBDD) is an approach that uses the 3D crystal structure of a drug target protein—typically obtained from X-ray crystallography, NMR, or cryo-EM—to computationally screen candidate molecules before any physical synthesis or wet lab work is performed [2]. This approach is based on a simple but powerful idea: if we know the exact 3D geometry of a protein's binding pocket—which atoms are where, which residues are polar, which are hydrophobic—then we can computationally simulate how well a given molecule will fit into that pocket and how strong the binding will be. The standard way it works with SBDD is as follows: First you have to obtain a high-resolution crystal structure of the protein of interest, typically through the RCSB Protein Data Bank. Then you should draw up this structure to be used in the computations - that is, to eliminate unnecessary parts, to insert hydrogen atoms, and to assign partial charges to the atoms. Then you delimit a grid box of the site where the protein binds other molecules. Then, you take a computer and attempt to dock a library of potential molecules with this grid, trying all the shapes that each molecule can assume and computing the affinity of each to the binding site. Better fitting molecules are then taken a closer look at, again, based on the binding energy score. The greatest advantage of the approach is that it is indeed quick. Assuming that you had to test 2,500 compounds in a real laboratory, this would require a great deal of time - several months - and a lot of money, millions of dollars. However, with computers, it is possible to perform the same in a few hours. This enables scientists to examine more chemicals and then just select the most favorable ones to experiment in the laboratory. It has now become a normal process of new medicines being discovered in the pharmaceutical industry.

This thesis employs the SBDD method to test the natural sesquiterpene compounds on the LasR QslA complex of Pseudomonas aeruginosa infection *Pseudomonas aeruginosa*. The focus is primarily structural—exploiting the exact geometry of the autoinducer-binding pocket of this complex to identify a perfect-fitting competitive inhibitor.

1.2 The need for an ideal drug target: the LasR–QslA Complex

An ideal drug target is one that: (a) clearly plays an important role in disease pathophysiology; (b) has a well-defined and tractable binding pocket that small molecules can target; (c) has no homolog in the human host or is very dissimilar so that selectivity is possible; and (d) is structurally resolved at high resolution so that structure-based design is possible. The LasR–QslA complex satisfies all these criteria [1,4]. The las quorum sensing (QS) system in P. aeruginosa is the apex of a hierarchical communication network. LasR is a LuxR-family transcriptional activator protein that dimerizes upon binding the cognate autoinducer 3-oxo-C12-HSL and activates a broad suite of virulence genes. LasI synthesizes the autoinducer. When the extracellular concentration of the autoinducer crosses a threshold, LasR is activated—and subsequently elastase, protease, exotoxin A, pyocyanin, and biofilm matrix genes are all turned on [1].QslA—Quorum Sensing-like Antiactivator—is a P. aeruginosa-specific negative regulator that binds directly to the ligand-binding domain (LBD) of LasR as a homodimer. It physically blocks the dimerization interface of LasR—locking LasR in the 'off' state even when autoinducer is present. Fan et al. (2013) determined the crystal structure of the LasR–QslA complex at a resolution of 2.3 Å in 2013. The structure was later deposited in the Protein Data Bank with the accession code 4NG2 [1], which was used as the structural basis for the computational analysis carried out in this thesis.

The 4NG2 structure is considered highly suitable for structure-based drug design because it contains a clearly defined and experimentally validated binding pocket where the native autoinducer binds accurately. When a second compound can fill this pocket better, either by forming better hydrogen bonds, or by having better geometrical fit or lower binding energy, it can disrupt LasR activity and inhibit the virulence signaling pathway. It is this basic idea that is the focus of the current research.

1.3 Autoinducer-Binding Pocket: Target Architecture

antiparallel beta-sheet (S1-S5) that holds the nucleotide, and a pocket that has residues of two functional classes: polar (hydrogen bond donors/acceptors) Tyr56, Asp73, Thr75, Val76, and Ser129 (forming the specificity layer of the pocket, which recognizes the polar groups of a potential inhibitor), and apolar Leu3, Val4, Phe7, Leu30, Val83, Leu84, Pro85, Leu148, Pro149, and Trp152 (forming the volume layer of the pocket, which defines

constraints on molecular size and hydrophobic character). One of the typical features of this pocket is that is highly buried in the protein core. The binding pocket is usually modeled to bind compact, globular, and multi-directional, with the polar amino acid residues, inhibitors. These structural requirements are well met by sesquiterpenes, C15 terpenoids with a backbone, which occur naturally [4,5].

The conformational dynamics have also been studied and the pocket has demonstrated to be not entirely rigid. Rather, it experiences small-scale structural variations that affect its shape and volume accessible. According to Manu et al., the active site of LasR has a high degree of flexibility and that aspect is significant in the choose of the success of an inhibitor [5]. This implies that an optimal inhibitor ought to be capable of keeping constant interactions despite fluctuations in the binding pocket in conformation. This is why compounds that can form more than one hydrogen bond are usually viewed as more favorable than the ones that are based on a single interaction.

1.4 Why Sesquiterpenes? Pharmacophoric Rationale

Sesquiterpenes are terpenoid compounds that are C15 and formed via the mevalonate and methylerythritol phosphate biosynthetic pathways. The term “sesqui” means “one and a half,” indicating that these molecules are structurally intermediate between C10 monoterpenes and C20 diterpenes. Distinct chemical changes like cyclization, hydroxylation, and oxidation of their isoprene units induce astounding structural variety yet still have a molecular weight range that is typically suitable as orally active drugs [8].

Sesquiterpenes also have various valuable pharmacophoric benefits. Its relatively low molecular weight is one of its main advantages in that they are typically between 200-300 g/mol. This is far below the upper limit of 500 g/mol recommended by Lipinski, meaning that such compounds have a higher chance of having good membrane permeability and good drug-like characteristics.

Second: Rigid ring systems (bicyclic or tricyclic) Geometric relationships of functional groups in a variety of conformational states are maintained - this offers predictable binding behavior. Third: Hydroxyl, carbonyl and ether groups have the potential to produce hydrogen bonds, thus allowing hydrogen bonds to interact with polar active sites [8,9]. Beta-caryophyllene, which is a bicyclic sesquiterpene, is already a validated reference inhibitor within LasR [6]. Its affinity -5.5 kcal/mol with LasR -5NG2 shows that the sesquiterpene scaffold can penetrate this cavity. But beta-caryophyllene has a TPSA of 0.00 Angstrom squared - that is, there are no polar functional groups - and its binding values are entirely hydrophobic [6]. This implies that the polar residues can be bound by the addition of polar substituents to the sesquiterpene scaffold hence increasing binding affinity dramatically.

An eremophilane-type sesquiterpene is called Glomeremophilane B (PubChem CID: 139589801). The eremophilane skeleton contains a 5-7 fused ring system- this pseudo-guanine carbon structure is quite rigid and encompasses several oxygen-carrying substituents. These oxygen containing functional groups present two possible hydrogen bond donor/acceptor sites, which can interact with the polar residues found at the LasR binding pocket. Due to this characteristic, glomeremophilane B is 2-3 times different than 8-caryophyllene and can have stronger and more resistant binding interactions with LasR [8].

1.5 Research Gap and Thesis Objectives (skip for now)

Previous studies on LasR virtual screening have examined different natural compounds, but the vast majority of this research has focused on the quorum sensing inhibition on a more general basis, or has focused more specifically on a subset of compounds, e.g., flavonoids and phenolics [10,11]. A comprehensive structural analysis specifically against the LasR–QslA complex (4NG2) of the eremophilane sesquiterpene class has not yet been performed. Furthermore, only one study has specifically screened sesquiterpene inhibitors using 4NG2 as a computational target [6].

The objectives of this thesis are: (i) to computationally prepare LasR–QslA (4NG2) for molecular docking; (ii) to screen a library of 2,500 natural compounds using AutoDock Vina; (iii) to perform a detailed structural analysis of the binding interactions of glomeremophilane B in BIOVIA DS; (iv) to structurally prove that the eremophilane sesquiterpene scaffold achieves a pharmacophoric fit for the LasR autoinducer pocket that is superior to the native autoinducer; and (v) to thoroughly validate the computational pipeline.

CHAPTER 2: LITERATURE REVIEW

2.1 Structure-Based Virtual Screening: A Tool Review

Structure-based virtual screening (SBVS) is a computational approach that uses the 3D structure of a target protein to systematically evaluate large compound libraries to identify compounds that best fit into the binding pocket. This approach has its roots in the 1980s and 1990s when the first computer-aided drug design tools were developed, but the real evolution of modern high-throughput SBVS occurred in the 2000s when computational power became affordable and the crystal structures available in the PDB increased exponentially [2,3]. Sliwoski et al. explained the basic principles of structure-based virtual screening (SBVS) in a detailed review. The central concept behind SBVS is that protein–ligand binding is an exergonic process, meaning the free energy of binding is negative. A more negative binding energy generally indicates a stronger interaction between the protein and ligand.

During docking, the algorithm generates several possible orientations and conformations of a ligand within the binding pocket. The scoring functions are then used to evaluate each pose with scoring functions that approximate binding affinity by combining weighted averages of various interaction parameters [2]. The compounds as potential hits are those that have the highest docking scores and are given precedence in further experimental studies.

The right definition of the docking grid is also an important measure of SBVS. The grid box is placed in the area of the active site based on the structural characteristics of the binding cavity. In case the grid is small, the ligand might not fully explore its possible conformations. Alternatively, too big of a grid may raise the chances of acquiring non-specific or false-positive binding. The perfect docking arrangement is a system where the grid box is perfectly centered based on the coordinates of the native ligand such that the whole binding cavity is well covered [3].

Another key step to a good SBVS study is protocol validation. One approach that is often applied is redocking a known inhibitor with a reported binding affinity experimentally under the same docking conditions. When the published result is similar to the predicted binding

value, the grid parameters and docking protocol are deemed to be reliable. In the present study, β-caryophyllene was selected as the reference compound for validation, and its reported binding energy of −5.5 kcal/mol was successfully reproduced [6].

Lionta et al. provided an in-depth overview of the principles, applications, and also the recent advancements in SBVS, such as scoring functions, docking, and enhanced hit identification rate strategies [3]. In their work, it was stressed that one of the most important factors that affect docking accuracy are the flexibility of ligands and the strength of polar interactions. The present work is particularly interested in these observations to interpret the docking behavior and binding performance of Glomeremophilane B.

2.1.1 Scoring Functions: How Bond Energy is Calculated

Scoring functions are a key component of computational docking and estimate the binding affinity of a ligand to its target protein. These scoring functions can be broadly categorized into three main groups: force-field based scoring functions, which are based on physical interaction energies, like electrostatic and van der Waals forces; empirical scoring functions, which are based on experimentally known protein-ligand interactions; and knowledge-based scoring functions, which are based on statistical information available in crystal structure databases.

The docking program (AutoDock Vina) utilized in this research study will utilize hybrid empirical scoring function which gives a good balance between the speed of calculations and accuracy of prediction [7].

Trott and Olson demonstrated that AutoDock Vina is equally as good as a number of commercial docking packages on common benchmarking datasets, and it is free to be used by academics [7]. One of the key enhancements that were made in their work was the use of a gradient-based optimization strategy, which enables the software to compare the multiple ligand conformations at the same time. This approach enables extensive conformational sampling without causing a dramatic increase in computational cost. An important limitation—which must be acknowledged when interpreting the results of this thesis—is that AutoDock performs docking without a rigid receptor by default. Protein conformation remains fixed during the docking process (only the ligand is flexible). This differs from the real biological scenario where a protein can adjust its conformation in response to a ligand. This does not capture 'induced fit' effects. This limitation justifies the need for future molecular dynamics simulations [5,7].

2.1.2 PyRx: Platform of High-Throughput Screening

PyRx (Python Prescription) is an open-source virtual screening platform that wraps AutoDock Vina in an intuitive graphical interface—making it accessible to efficiently screen large compound libraries. Mun et al. (2024) reviewed the use of PyRx in computational drug discovery and demonstrated that the platform is validated and widely used to screen thousands of compounds in a systematic workflow.[12]. PyRx workflow: import SDF files, perform energy minimization using Universal Force Field (UFF), convert to PDBQT format, load receptor file, define grid parameters, and run batch docking. Output is a ranked list with binding affinities. This ease of use has made PyRx a widely adopted tool in academic drug discovery research, and in the present study it was successfully used to screen a library of 2,500 compounds.

2.2 LasR Structural biology: What is known so far

The understanding of LasR structural biology has advanced considerably over the past decade. Several crystal structures of LasR are now available in different conformational states, including autoinducer-bound, agonist-bound, and partial agonist-bound forms. Collectively, these structures give comprehensive understanding of the conformational landscape/functional flexibility of the receptor [1,4]. In their landmark study of 2013, Fan et al. finally worked out the crystal structure of the LasR-QslA complex (PDB: 4NG2). In their work, they demonstrated that QslA binds to the dimerization interface of LasR and that this is the same area that is necessary to bind the second LasR monomer when forming a dimer. Notably, this is achieved following the binding of autoinducer. QslA can still suppress productive dimerization; even in the presence of the pre-existing association of the autoinducer with LasR, it can bind to the stabilized monomer [1]. Such mechanistic understanding is of great importance to drug design since it implies that inhibition of the ligand-binding domain (LBD) cavity might replicate the endogenous inhibitory effect of QslA. The functionality of hydrogen bonding in the LasR LBD has also been discovered in details. Past studies have put the coefficient of various hydrogen bond interactions between LasR and its autoinducer in order of rank, with certain polar contacts being vital to ligand recognition and receptor activation [13]. Of these, the S129 residue has been found to be especially significant as it is able to form a hydrogen bond with the lactone head group of the native autoinducer and also as a driving force to ligand selectivity. The importance of several polar interactions is directly connected with the results of the current research. Glomeremophilane B was found to react with the residues TRP75, TRP76, THR76 and GLN103, all of which belong to the important polar interaction network of LasR ligand-binding domain. The relevance of conformational flexibility of LasR was also examined by Manu et al. who showed that the active site is not structurally rigid. Instead, it is the receptor that experiences changes in conformation that greatly affect the inhibitor recognition and binding behavior[5]. 'Breathing motions' of the binding pocket dynamically modulate the accessible volume and shape. This finding is practically

important: it means that a compound that establishes multiple H-bond contacts with LBD polar residues—such as glomeremophilane B—will be more robust in maintaining binding across conformational fluctuations compared to single-contact inhibitors. A recent study on patuletin (a natural methoxyflavone) demonstrated a -20.96 kcal/mol binding affinity with LasR using MOE software, and 100 ns molecular dynamics confirmed that the complex was stable and that specific hydrogen bonding with key amino acids was maintained [14]. This study demonstrates that natural compounds can form extraordinarily stable complexes with the LasR LBD—and specific in vitro validation also confirms computational predictions [14]. Virtual screening of FDA-approved drugs has also been performed against LasR — sulfamerazine showed the highest binding affinity and 50 ns MD simulations demonstrated a stable binding conformation [15]. Molecular docking and MD simulation studies with quercetin identified that this compound can adopt multiple binding modes with the LasR LBD — and principal component analysis suggested that the LBD-binding mode is most relevant [16]. These studies collectively establish that the LasR LBD is a genuinely druggable target for diverse chemical scaffolds.

2.3 Previous Docking Studies on LasR: what has happened

The literature of virtual screening studies against LasR is now considerable. Magri et al. (2023) targeted the LasR receptor with food-derived natural compounds and reported affinities exceeding -13 kcal/mol—establishing that an extraordinary range of LasR-complementary structures are available in natural product libraries [10]. This study also has important differences from the current work: it uses a different LasR crystal structure and does not explicitly target the QslA complex structure.i.. Azzouni et al. (2025) conducted a comprehensive phytochemical analysis of Vitex agnus-castus essential oil in which beta-caryophyllene — a dominant sesquiterpene — demonstrated a -5.5 kcal/mol affinity against the LasR–QslA complex (4NG2) [6]. This paper will offer a first hand basis to the current paper that directly confirms 1-caryophyllene as a credible reference standard. The work by Azzouni et al. was however constrained with a relatively small number of phytochemicals and was not an extensive screen of a larger sesquiterpene library against the 4NG2 structure.

Bhaskar et al. used a pharmacophore-based structure-guided virtual screening against the Pseudomonas aeruginosa infection P. aeruginosa LpxA protein. Their results showed that natural compound screening is effective to be utilized concerning several therapeutic targets in the organism [17]. This gives credence to the notion that a number of proteins of P. aeruginosa have good drugs queasable properties to drugsqueasable structure-based virtual screening (SBVS) methods.

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Manu and others also used the dynamics of conformational changes in the LasR receptor and demonstrated that the adaptability of the active site contributes significantly to the potency of inhibitors [5]. In their work it is emphasized that being able to dock with high affinity is not sufficient to be an effective inhibitor but a successful one should be able to preserve the same interactions with the receptor throughout the various conformational states of the receptor.

Such results are directly applicable to the analysis of hydrogen bond-dominant interaction pattern of glomeremophilane B. LasR inhibitors have been discovered using machine learning methods as well. A data-driven ML approach identified novel LasR inhibitors and confirmed ligand stability from 200 ns MD simulations [18]. This work demonstrates that the computational drug discovery field is evolving—from molecular docking to molecular dynamics and now machine learning—and future MD validation for glomeremophilane B is a natural next step in this trajectory.

2.4 Sesquiterpene Scaffolds in Drug Discovery

Sesquiterpenes are a historically productive compound class in natural product drug discovery. Artemisinin — the Nobel Prize winning antimalarial drug — is a sesquiterpene lactone. Parthenolide (anti-inflammatory), guaianolide class sesquiterpenes (anti-cancer), and multiple sesquiterpene alcohols (antimicrobial) — all demonstrate that the C15 terpenoid framework is capable of diverse biological activities [8,9]. Eremophilane-type sesquiterpenes are specifically isolated from marine-derived fungi and endophytic fungi. Zhang et al. (2022) isolated copteremophilanes A-J from the marine-derived fungus Penicillium copticola — these compounds showed antitumor and neuroprotective activities [9]. This establishes that the eremophilane class is a biologically active framework against diverse targets. Yang et al. (2025) isolated eremophilane sesquiterpenoids from the marine-derived fungus Phoma sp. that show anti-neuroinflammatory activity, and computational docking studies suggested a potential interaction with the iNOS protein [19]. This recent work demonstrates that eremophilane sesquiterpenes can produce relevant hits in computational docking studies against protein targets—a finding that is directly relevant in the context of the docking of glomeremophilane B to LasR. The novel eremophilane sesquiterpenes were identified as a product of the marine sediment-derived

fungus Emericellopsis maritima, and these were cytotoxic and antimicrobial [20]. Taken together, these studies are indicative that the eremophilane scaffold has a significant pharmacological potential and can generate biologically active compounds exhibiting antimicrobial properties. These results indicate that glomeremophilane B may be a biological plausible LasR inhibitor.

2.5 Computational Tools: PyRx, AutoDock Vina, BIOVIA DS — A Technical Review

This study used three of the key computational tools and their technical principles should be known so that the results may be interpreted properly.

AutoDock Vina is an open source docking engine created by Trott and Olson [7]. Vina docking algorithm applies the gradient-based optimization algorithm with the series of independent docking runs that allows the exploration of a number of potential ligand-binding conformations in one simulation. The software provides binding affinity values in kcal/mol, with more negative values indicating stronger predicted binding interactions.

AutoDock Vina has a scoring function that considers intermolecular and intramolecular binding contributions to the computation of a score. Intermolecular interactions are steric contacts, hydrogen bonding, and electrostatic interactions between the ligand and the protein, whereas in intramolecular terms, one can consider factors like torsional entropy due to ligand flexibility.

PyRx Platform: AutoDock Vina is accessed through the PyRx interface [12]. PyRx's Open Babel integration automatically handles SDF-to-PDBQT conversion. Energy minimization in PyRx uses the Universal Force Field (UFF) which makes parameters available for all elements — important because natural compound libraries contain diverse chemistries. The exhaustiveness parameter is set to 8 for standard throughput — increased to 16 when needed in refined runs for top compounds. BIOVIA Discovery Studio Visualizer 2024: This is the industry-standard platform for post-docking interaction characterization [21]. Baroroh et al. (2023) published a detailed protocol for using BIOVIA DS for molecular interaction analysis and visualization—describing how 2D ligand interaction diagrams and 3D surface-bound complex visualizations are generated [21]. BIOVIA DS can identify conventional hydrogen bonds, Pi-Pi stacking, hydrophobic contacts, van der Waals forces, and carbon-hydrogen bonds and presents them in color-coded 2D maps that provide publication-quality

figures. Open Babel: Open Babel—an open chemical toolbox for ligand structure preparation—was used to convert SDF files to PDBQT format [22]. O'Boyle et al. (2011) described the capabilities of Open Babel—including Gasteiger charge model application and identification of rotatable bonds—both steps that are critical for accurate docking without AutoDock [22].

CHAPTER 3: MATERIALS AND METHODS

3.1 Protein Structural Refinement

The 3D crystal structure of the P. aeruginosa LasR–QslA complex was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org) under PDB accession code 4NG2 [1]. This structure is solved at 2.3 Angstrom resolution—using a combined approach of molecular replacement and single-wavelength anomalous dispersion (SAD) phasing—and crystallizes in the P222 space group. The asymmetric unit contains four independent LasR–QslA assemblies, each one containing the monomeric LasR LBD (residues 1–170) co-crystallized with the autoinducer 3-oxo-C12-HSL, and two QslA subunits in 2:1 stoichiometry. of the autoinducer. The raw PDB structure file was visually analyzed using BIOVIA Discovery Studio Visualizer 2024 to identify the spatial boundaries of the binding pocket. The binding location is directed by the coordinates of the co-crystallized ligand 3-oxo-C12-HSL. Cleanup protocol Structural Crystallographic water molecules were eliminated; heteroatoms were eliminated; biologically irrelevant protein chains were eliminated. Chain E (LasR LBD) and Chain F (QslA dimer) were only retained because they maintain the native protein-protein interface [1]. Ambiguous structures that had poor electron density were also eliminated structurally. The last structure that had been prepared was exported to the PyRx platform to the PDBQT format, which is the input format required by AutoDock Vina by means of assigning Gasteiger partial charges and adding polar hydrogens.

3.2 Ligand Library and Preparation

PublChem database (https://pubchem.ncbi.nlm.nih.gov) was used to compile a natural compound library. The physicochemical filtering criteria of this study were chosen in order to obtain compounds with good drug-like properties prior to docking analysis. The applied parameters were 300-400 g/mol molecular weight range, 0-5 hydrogen bond donors, 0-10 hydrogen bond acceptors with topological polar surface area (TPSA) 20-140 a2 and 40 heavy atoms. These criteria are in line with the Lipinski 5 rule [23] and aid in filtering out compounds with poor drug-like properties before computationally intensive docking programs are run.

In this space of filtered compounds, the sesquiterpene-based natural products were targeted due to their structural compatibility with LasR binding pocket. Eremophilane sesquiterpene Glomeremophilane B (PubChem CID: 139589801), a compound with several oxygen-carrying functions, was selected as a compound of interest and β-caryophyllene which is the most common sesquiterpene in Vitex agnus-castus essential oil and has been previously characterized against the 4NG2 structure (ref) [6] was a validated reference inhibitor. An inclusion of the native autoinducer 3-oxo-C12-HSL was also used as an example of biological control.

The structures of the ligands were collected in PubChem in SDF format. Ligand files were converted to PDBQT format with Open Babel [22], Gasteiger charges have been assigned to permit compatibility with AutoDock Vina scoring system. Energy minimization

was done before docking with the Universal Force Field (UFF) inside the PyRx interface. About 2,500 compounds were in the final screening library.

3.3 Grid Box Configuration and Docking Setup

The AutoDock Vina program via the PyRx package (0.8) was used to perform molecular docking. A correct positioning of the docking grid box was one of the most significant methodological steps in the study. The co-crystallized native ligand, 3-oxo-C12-HSL, was precisely centred in the grid by using the three-dimensional coordinates of the native ligand-binding cavity of the LasR ligand-binding domain (LBD). Final grid box parameters: Center X = 31.281, Y = 10.4992, Z = 21.7813. Dimensions: X = 25.000, Y = 25.000, Z = 25.000 Angstrom. These grid dimensions completely covered the LasR autoinducer-binding cavity and provided sufficient space for thorough conformational sampling during docking. This binding pocket is found in the first 170 residues of LasR ligand-binding domain (LBD) and has a structural organization around a five-strand antiparallel 8. The active site hosts significant polar residues that include TRP75, TRP76, THR76, and GLN103, which are primarily involved in hydrogen bonding interactions and hydrophobic ones, including LEU99, ALA79 and VAL96, which are involved in van der Waals interactions.

To screen the entire library of compounds virtually, the exhaustiveness parameter of AutoDock Vina was used to 8 whereas a greater parameter of 16 was employed to further analyze the top-ranked compounds.

To confirm the docking protocol, β-caryophyllene was re-docked under the same experimental conditions. The affinity of the reproduced binding with the value of -5.5 kcal/mol was identical to the one published earlier [6], and it verified the reliability and methodological correctness of the docking configuration.

3.4 Post-Docking Interaction Analysis

Post-docking structural analysis was performed using BIOVIA Discovery Studio Visualizer 2024 [21]. Detailed interaction fingerprint analysis was carried out by separately importing the top-ranked docking poses of Glomeremophilane B, β-caryophyllene, and the native ligand 3-oxo-C12-HSL. The analytical workflow followed the methodology described by Baroroh et al. [21].

The interaction analysis included a wide range of non-covalent interactions, such as conventional hydrogen bonds with distance criteria of 2.5–3.5 Å, Pi-donor hydrogen bonds, Pi-anion electrostatic interactions, Pi–Pi stacking and T-shaped interactions, Pi-sigma interactions, carbon–hydrogen bonds, van der Waals interactions, and hydrophobic contacts.

Both two-dimensional ligand interaction maps and three-dimensional surface-bound complex visualizations were generated for detailed interpretation. Interaction assignments at the residue level were recorded, and both qualitative and quantitative differences among the three ligands were systematically compared [1].

3.5 ADME Evaluation — Structural Context

Pharmacokinetic properties and drug-likeness profiles were evaluated using the SwissADME web server. Although the main focus of this thesis is structural docking analysis, ADME evaluation was included to support the structural findings by assessing

whether the scaffold of Glomeremophilane B also demonstrates favorable drug-like characteristics for potential clinical application.

SwissADME is a pharmacokinetic prediction platform created by Daina et al. at the Swiss Institute of Bioinformatics and that relies on a proven QSAR model [24]. The parameters assessed were molecular weight, consensus Log Po/w which is used to evaluate lipophilicity, topological polar surface area topographical polar surface area (TPSA), gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein substrate prediction, inhibitory activity of five major CYP isoforms, bioavailability score, and synthetic accessibility (SA) score.

The drug-likeness was evaluated as per the Rule of Five by Lipinski [23]. PubChem retrieved the SMILES representations of the compounds and fed them to SwissADME.

CHAPTER 4: RESULTS

4.1 Thermodynamic Scoring — Binding Affinities

After the systematic virtual screening with 2,500 natural phytochemicals in a library against LasR-QslA autoinducer-binding pocket (PDB: 4NG2), a wide spectrum of binding affinities was found among the screened compound. Table 4.1 is a summary of the binding affinities of the best candidates.

Table 4.1: Top-Ranked Compounds — Binding Affinities Against LasR–QslA Complex (4NG2)

Rank	Compound	PubChem CID	Binding Energy (kcal/mol)
1	Glomeremophilane B (Lead)	139589801	−7.9
2	Penicidone A	—	−7.6
3	N-formyllapatin A	—	−7.5
4	Guignardone C	—	−6.8
5	Photinide E	—	−6.2
6	Trichocladinol B	—	−5.9
7	Nemanolone C	—	−5.8
—	Beta-caryophyllene (Protocol Validator)	5281515	−5.5
—	3-oxo-C12-HSL (Native Autoinducer / Control)	—	−5.2

Protocol validation confirmed by redocking beta-caryophyllene — reproduced affinity -5.5 kcal/mol exactly Azzouni et al. (2025) [6]. These results validate the docking protocol and grid parameters employed in the experiment as methodologically sound and able to identify real differences in structural complementarity in the screened compounds.

The best hit at the binding affinity of -7.9 kcal/mol was glomeremophilane B (PubChem CID: 139589801) 2.4 kcal/mol stronger than 8-caryophyllene and 2.7 kcal/mol stronger than the native autoinducer. Chemical thermodynamically, a difference in the binding constant of about 1.36 kcal/mol at 298 K corresponds to a change in predicted binding constant of almost ten times. According to this relationship, the binding affinity of Glomeremophilane B is predicted to be about 100-fold higher than the native autoinducer, which means that there would be a significant benefit in competitive displacement in the LasR binding pocket.

4.2 Glomeremophilane B’s Interaction Fingerprint — Structural Analysis

A more in-depth post-docking analysis of the highest-rank binding pose of Glomeremophilane B in the BIOVIA Discovery Studio Visualizer indicated the existence of a large multi-point hydrogen bond network in the LasR ligand-binding domain (LBD).

The compound formed six conventional hydrogen bonds:
(i) TRP75:N at a distance of 2.14 Å,
(ii) TRP76:N at 2.23 Å,
(iii) THR76:OG1 at 2.74 Å,
(iv) GLN103:NE2 at 3.23 Å,
(v) THR100 at 2.91 Å, and
(vi) GLY72 at 3.10 Å.

In addition, a carbon–hydrogen interaction was observed with LEU99. Together, these interactions create a highly organized polar interaction network within the LasR binding cavity.

Three-dimensional surface visualization further confirmed that Glomeremophilane B fits effectively within the autoinducer-binding pocket. The geometry of its eremophilane ring system showed strong spatial complementarity with the cavity formed around the five-stranded β-sheet structure of the LBD. The two-dimensional interaction map also demonstrated that the majority of the polar interactions were concentrated within the defined polar region of the LasR binding site, corresponding to the same region where the lactone head group of the native autoinducer normally interacts.

The observed interaction profile, consisting of six conventional hydrogen bonds along with an additional carbon–hydrogen interaction, indicates a highly dense and stable binding arrangement that appears structurally more favorable than that of the native autoinducer. The interactions involving TRP75 and THR76 are especially significant because Manu et al. identified these residues as important contributors to the structural stability and functional dynamics of the LasR active site [5].

4.3 Interaction Profile of Native Autoinducer — Structural Comparison

The interface profile of the natural ligand 3-oxo-C12-HSL is evidently different from that of Glomeremophilane B. 3-oxo-C12-HSL forms: a conventional H-bond with ARG F:82:NH2; a Pi-donor H-bond with TRP75; a carbon-hydrogen bond with VAL76. Hydrophobic contacts: with VAL96, ALA79, Leu99, TRP75, and PHE78 [1]. Binding energy: -5.2 kcal/mol. An important structural observation from the docking analysis is that binding of the native autoinducer is mainly driven by hydrophobic interactions. Its long C12 acyl chain is seen to occupy a hydrophobic site of LasR binding pocket which comprises of Leu3, Val4, Phe7, Val83, Leu84, and Pro85 residues. Conversely, polar ligand-binding domain (LBD) residues interactions are weak with only one conventional hydrogen bond being found between Arg82.

This comparatively restricted pattern of polar interaction symbolizes a structural constraint that Glomeremophilane B seems to conquer by means of the creation of various hydrogen bonds in the binding cavity.

The behavior of the interaction profile of β-caryophyllene was different. No standard hydrogen bonds were found which is not surprising by the fact that its TPSA value is 0.00

A 2 and it does not have any polar functional groups. Its binding specificity of -5.5 kcal/mol thus is almost purely hydrophobic and van der Waals interactions. This largely hydrophobic mode of interaction is likely to be less specific as well as less structurally stable compared to the large multi-point hydrogen bonding network established by Glomeremophilane B.[6].

4.4 ADME Profile — Structural Context

Table 4.2 SwissADME-derived parameters are presented in. In this section, the ADME data are interpreted from a structural angle—to show that the scaffold of glomeremophilane B is clinically viable.

Table 4.2: SwissADME Comparative Analysis — ADME aur Drug-Likeness Parameters

Parameter	Glomeremophilane B	Beta-caryophyllene	3-oxo-C12-HSL
Molecular Weight (g/mol)	292.33	204.35	297.39
Consensus Log P	1.51	4.24	2.93
TPSA (Å²)	72.83	0.00	72.47
GI Absorption	High	Low	High
BBB Permeant	No	No	Yes
P-gp Substrate	No	No	No
CYP1A2 Inhibitor	No	No	Yes
CYP2C19 Inhibitor	No	Yes	Yes
CYP2C9 Inhibitor	No	Yes	No
CYP2D6 Inhibitor	No	No	No
CYP3A4 Inhibitor	No	No	No
Lipinski Violations	0	1	0
Bioavailability Score	0.55	0.55	0.55
Synthetic Accessibility	4.84	4.51	3.05

Key structural-ADME connection: Glomeremophilane B has a TPSA of 72.83 Angstrom squared—this comes directly from its oxygen-bearing substituents establishing H-bonds with polar residues in the binding pocket. Meaning, the same structural feature that facilitates hydrogen bonding (polar functional groups) also ensures high GI absorption and good aqueous solubility in ADME. Both of these properties are simultaneously optimized by an integrated structural feature—this is not a coincidence, it is an inherent advantage of the sesquiterpene scaffold.

The zero TPSA of beta-caryophyllene directly explains its poor GI absorption—no polar groups mean no hydrogen bonding capacity and poor aqueous solubility. This ADME failure was structurally expected — for the same reason that the LasR docking result was poor: hydrophobic only scaffold.

CHAPTER 5: DISCUSSION

5.1 Conformational Fit and Binding Mode Superiority — Structural Argument

The main structural finding of this thesis is that Glomeremophilane B exhibits a more favorable conformational fit within the LasR–QslA autoinducer-binding pocket than the native ligand 3-oxo-C12-HSL. This improved fit appears to arise from the geometry of its eremophilane scaffold, which enables the simultaneous formation of multiple polar interactions with important residues of the ligand-binding domain (LBD).

This interpretation can be understood by examining the structural organization of the LasR binding pocket. The ligand-binding domain contains a five-stranded antiparallel β-sheet that forms an internal cavity organized into two major interaction regions. The upper region is primarily polar in nature and includes residues such as TRP75, TRP76, THR76, and GLN103, which participate in hydrogen bonding interactions. The lower region is predominantly hydrophobic and contains residues including LEU99, ALA79, VAL96, and PHE78, which accommodate the long acyl chain of the native autoinducer through hydrophobic interactions [1].

The native autoinducer 3-oxo-C12-HSL primarily occupies the hydrophobic layer — its long acyl chain sits in the apolar channel and forms only one H-bond with the polar head group Arg F:82. This binding mode is 'hydrophobic dominant, polar-minimal'. Structurally, this is not optimal — because the polar layer remains largely unexploited.

Glomeremophilane B exhibits a fundamentally different binding pattern compared to the native autoinducer. Its compact eremophilane ring system, characterized by a fused 5–7 membered C15 framework, fits effectively within the polar region of the LasR ligand-binding domain. The arrangement of its oxygen-containing substituents allows them to adopt a favorable spatial orientation for simultaneous hydrogen bonding interactions with TRP75, TRP76, THR76, and GLN103.

This predominantly polar mode of interaction appears structurally more favorable than binding modes that rely mainly on hydrophobic occupancy of the GLN103 region, as it promotes stronger and more specific stabilization within the binding pocket.

Why superior? There are three major structural reasons for this enhanced binding behavior.

To begin with, hydrogen bonds are strongly directional and energetically favorable interactions, which normally add about -1 to -5 kcal/mol/interaction. The six hydrogen bonds of Glomeremophilane B thus gives a cumulative stabilizing effect, which is significantly larger than the interactions mainly between van der Waals forces that can be seen with the native autoinducer.

Second, because of the interaction with the polar layer of LasR ligand-binding domain, the specificity of binding is improved. These hydrogen bonds engage specific amino acid

residues and the geometry is specific, which lowers the chances of non-specific or off-target interaction.

Third, multiple simultaneous contacts helps to enhance the kinetic stability of the protein–ligand complex. Although a single hydrogen bond may transiently break due to changes in conformation, the other interactions can still stabilize the ligand in the binding pocket, thus stabilizing the overall binding of the ligand [5].

A study of conformational dynamics conducted by Manu et al. (2025) is a key evidence supporting this argument. They demonstrated that the adaptability of the LasR active site is one of the determinants of inhibitor strength - and that, in particular, the interactions glomeremophilane B makes with other residues, including TRP75 and THR76, are what controls active site stability [5]. This would directly suggest that the binding conformation of glomeremophilane B will be dynamically stable- not only in the stationary crystal structure.

5.2 Scientific Significance of Protocol Validation

The attack on any computational study is most often the criticism: These are mere computer predictions--do they look like real binding? The most straightforward method of dealing with this objection is protocol validation protocols minimizing an experimentally determined inhibitor under the identical conditions and determining whether the value obtained is the same as the published value.

One of the reasons why beta-caryophyllene was used as the validation reference in this study is that: (1) Azzouni et al. (254) specifically docked beta-caryophyllene against 4NG2 at -5.5 kcal/mol [6]; (2) is a well-characterized compound whose LasR binding behavior had been experimentally and computationally described; and (3) is a sesquiterpene- the same compound class to which glomeremophilane B belongs- that controls consistent comparisons.

Exactly the same value was reproduced as the previously published value giving high methodological validation of the docking protocol used in this study. The significance of this discovery is this: since the protocol could reproduce accurately the known binding behavior of -caryophyllene, the calculated binding affinity of -7.9 kcal/mol that can be obtained towards Glomeremophilane B has a higher chance of reflecting true structural complementarity as opposed to a computational artifact.

Another interesting point is the uncharacteristically accurate coincidence of reproduced and published values. The AutoDock Vina scoring functional normally has a natural error of about ±0.5–1.0 kcal/mol. Thus, the fact that the docking grid was exactly matched implies that the docking grid was optimized, and the chosen exhaustiveness parameter

permitted that enough conformational sampling be performed when calculating the docking. This agreement goes further to enhance the confidence with which the greater docking performer is interpreted to be that of Glomeremophilane B [7].

5.3 Pharmacophore Implications: Sesquiterpene As Optimal Scaffold for LasR Inhibition

A significant broader observation of this experiment is that LasR autoinducer-binding pocket does seem to be structurally selective to scaffolds based on sesquiterpenes, especially those with oxygen-containing functional groups that are able to interact with the polar region of the binding site.

The docking outcome of this trend is supported by the docking results obtained in the current analysis where β-caryophyllene, which does not have polar functional groups, had a binding affinity of -5.5 kcal/mol whose interaction was primarily due to hydrophobic interactions. Conversely, Glomeremophilane B, with several hydroxyl-bearing substituents, resulted in a significantly higher binding affinity of -7.9 kcal/mol by using the multiple hydrogen bond-mediated interactions. The recorded difference of 2.4 kcal/mol thus seems to be closely linked with the availability of these polar functional groups.

Pharmacophore-wise, the findings suggest that future design of LasR inhibitors can be enhanced to have strategically located hydroxyl or carbonyl groups incorporated within sesquiterpene structures. These changes might increase the ability of the hydrogen-bonding with important polar residues of the ligand-binding domain and consequently increase the binding affinity and stability of the interaction.

Magri et al. found that numerous food-derived natural compounds were more strongly bound to LasR than -13 kcal/mol suggesting that natural scaffolds that are highly complementary to this receptor are present in natural compound libraries [10]. They however carried out their work with different LasR crystal structures.

The results of the current experiment along with the results of the screening of sesquiterpene library against the structure of 4NG2 indicate a regular structure in LasR ligand recognition. The binding pocket seems to be selective to compounds that:

(1) have a rough C15 molecular structure,

(2) include a small and somewhat rigid core design, and

(3) contain several polar substituents which can react with the polar location of the active site.

This proposed pharmacophore profile is similar to glomeremophilane B. It has a highly structured eremophilane framework with extensive oxygen-containing substituents and a fused 5-7 ring system, which is well positioned structurally to fit LasR binding cavity.

Due to these properties, the compound is a good lead scaffold to be used in future structure-activity relationship (SAR) studies.

Future SAR experiments may systematically vary the number and location of hydroxyl groups but retain the eremophilane core architecture to identify which patterns of substitution enhance hydrogen-bond dynamics in the LasR ligand-binding domain to the greatest extent.

Another benefit of this category of scaffold is the widespread biological effects that have already been reported of eremophilane sesquiterpenes, such as antitumor, neuroprotective, antimicrobial, and anti-neuroinflammatory activity [9,19,20]. Such observations indicate that the eremophilane framework can be used in interactions with a variety of protein targets and commonly is biologically compatible. Here, LasR inhibition is a new and potentially useful use of the well-characterized eremophilane scaffold.

5.4 Future Computational Directions

The results of this thesis are a solid foundation, both to experimental validation and more complex computational studies. The use of molecular dynamics (MD) simulations is one of the most significant future directions. Whereas molecular docking gives a more static view of the protein-ligand interactions at a given point in time, MD simulations enable one to study both the dynamics of the protein-ligand complex and the stability of the complex itself in conditions that are closer to the realistic physiological conditions.

One of the possible future directions would be to conduct a 100 500 ns classical molecular dynamics (MD) simulation of the Glomeremophilane B4NG2 complex with the help of GROMACS or AMBER packages. Protein parameterization could be done with the AMBER force field and ligand parameterization could be done with the GAFF2 force field. The TIP3P water model and physiological salt concentrations would be used to prepare the simulation system under periodic boundary conditions in order to recreate biologically relevant conditions. To monitor RMSD, RMSF, radius of gyration and hydrogen bond persistence in analysis. This information will reveal binding solidity and time-perseverance of contact.
Yadav et al. established that a 100 ns molecular dynamics simulation of the Patuletin-LasR complex identified that the complex was stable and hydrogen bonding interactions with amino acid residues of special significance during the simulation time [14]. Depending on the large amount of hydrogen-bonding network that is present in Glomeremophilane B in the present study, it is possible that the same amount of dynamic stability can be anticipated. Nevertheless, this prediction is yet to be substantiated by detailed MD simulations and experiment validation.
The binding free energy calculations of MM-PBSA or MM-GBSA binding are much more accurate estimates of binding affinity than MD trajectory static docking scores - by explicitly including solvation, conformational entropy, and protein flexibility. Such calculations will determine absolute binding free energies of Glomeremophilane B and other highest ranking compounds - again confirming the competitive ranking [3].
Another significant future direction of this work is ensemble docking where ligands are docked to multiple protein conformations that were produced during molecular dynamics simulations. This strategy considers the conformational flexibility of LasR active site reported by Manu et al. [5]. Ensemble docking can resolve one of the biggest problems of traditional docking methods by representing multiple receptor conformations, rather than a single fixed structure, and can offer realistic insights into the ligand binding behavior.

This can also explain and confirm the good binding performance of the Glomeremophilane B.

Moreover, scoring functions commanded by machine learning are also an encouraging new development to enhance docking precision. Jokinen et al. have shown the efficiency of machine learning-aided scoring schemes to assess LasR inhibitors [18]. The further use of these sophisticated scoring techniques on Glomeremophilane B may offer an extra measure of reliability on the predicted binding affinity and stability of the interaction to traditional docking calculations.

The other significant future direction that can come up as a result of this study is the creation of a pharmacophore model. Through examining the principle interaction fingerprints of the ten highest-ranked compounds such as the ones mentioned above; Glomeremophilane B, Penicidone A and N-formylapatin A, a common set of structural and interaction characteristics that are required to achieve effective LasR inhibition can be determined. The essential molecular characteristics that are obligatory to stabilize the binding in LasR ligand-binding domain can be determined by such a pharmacophore model, such as the spatial arrangement of hydrogen bond acceptors or donors, hydrophobic regions and rigid scaffold geometry. This model can subsequently be used to guide the design of rational compounds and screening of more chemical libraries, thereby aiding a more structure-directed process to subsequent drug optimization programs.

Through interaction fingerprint analysis of the top ranked compounds, e.g. Glomeremophilane B, Penicidone A and N-formylapatin A, we can possibly determine a set of structural and interaction features that the LasR is sensitive to in order to inhibit it effectively.

A pharmacophore model like this might map the key molecular features necessary to achieve stable binding into the LasR ligand-binding space, including the spatial distribution of hydrogen bond donors or acceptors, hydrophobic regions and rigid scaffold geometry. This model, once in place, could serve as a model to be followed in rational compound design and screening of additional chemical libraries and thereby enhance the approach of a structure-driven approach to future drug optimization programs.

CHAPTER 6: CONCLUSION

Systematic virtual screening of 2,500 natural phytochemicals were conducted against the autoinducer-binding pocket of the LasR–QslA complex (PDB: 4NG2) in this computational thesis. The main aim of the study was to identify a structurally effective competitive inhibitor, which could interact with the key polar residues of the binding pocket through the hydrogen-bonding interactions.

The most significant outcome of this research was the discovery of Glomeremophilane B (PubChem CID: 139589801) as the top-ranked compound with a binding affinity of −7.9 kcal/mol. This value was thermodynamically more favorable by 2.7 kcal/mol than the native autoinducer 3-oxo-C12-HSL and by 2.4 kcal/mol than the validated reference compound β-caryophyllene.

Furthermore, the methodology further validated the reliability of these findings. The docking protocol was able to reproduce the binding affinity of β-caryophyllene that has been previously reported at exactly −5.5 kcal/mol, the same as the published value, thereby confirming the robustness and accuracy of the docking setup used in this study [6]. The docking protocol was able to reproduce the previous binding affinity of β-caryophyllene at precisely −5.5 kcal/mol , which is exactly the published value , which confirms the robustness and accuracy of the docking setup used in this study [6].

The structural basis underlying the superior binding behavior of Glomeremophilane B was clearly demonstrated through interaction analysis. The compound was found to bind six traditional hydrogen bonds to the major polar residues of LasR ligand-binding domain, such as TRP75, TRP76, THR76, and GLN103. This large multi-point hydrogen-bonding interaction is far a more powerful and specific pattern of interaction than the weak polar interaction observed with the native autoinducer.

Three-dimensional visualization of surfaces and two-dimensional interaction mapping were additional confirmations that the glomeremophilane ring system perfectly fits the ligand-binding cavity of LasR. The geometry of the scaffold was complementary with the structural arrangement of the pocket and contributed to the high binding affinity and steady interaction profile [1].

An expanded pharmacophore understanding that comes out of this experiment is that sesquiterpene scaffolds, especially with numerous oxygen-bearing functional groups are highly structurally compatible with the LasR autoinducer-binding pocket. The difference between 2.4 kcal/mol in the binding affinity between 2- caryophyllene, which does not have any polar functional group, and Glomeremophilane B, which has several hydroxyl functional groups, seems to be directly related to the presence of polar functional groups. The observation offers a valuable structural template of what LasR inhibitors will be like in the future and how to optimize them [8].

The predicted ADME profile of Glomeremophilane B also favours its possibility as a drug-like scaffold. The compound had TPSA of 72.83 A 2, log P of 1.51, high gastrointestinal absorption, no CYP inhibitions predicted, and complied perfectly with the Lipinski Rule of Five. Notably, the oxygen-containing substituents which contribute to these desirable pharmacokinetic characteristics are the functional groups that are involved in hydrogen bonding in LasR binding cavity. This implies that both the strong structural binding

interactions and the favorable pharmacokinetic properties are made in the same integrated molecular features with the eremophilane scaffold of Glomeremophilane B [23,24].

The thesis is the first computational study of Glomeremophilane B targeting LasR -QslA complex (PDB: 4NG2) in particular. According to the in silico results of this paper, the eremophilane sesquiterpene skeleton of Glomeremophilane B can be considered as a good lead framework in the future LasR-targeted discovery of medicines.

The results of the computations form a good basis to further support the results using more intensive means of computation like molecular dynamics (MD) simulations, MM-PBSA free energy calculations, ensemble docking studies. In addition, subsequent in vitro experimental investigations will be essential to confirm the predicted inhibitory potential and biological activity of the compound.

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